

PATENT
ATTORNEY DOCKET NO.: UCSD1130-1

Applicants: Fahey, et al.

Application No.: 09/530,370

Filed: July 6, 2000

Page 7

REMARKS

Claims 1-50 were pending prior to this response, with claims 12-39 and 43-45 being withdrawn in response to a restriction requirement herein. By the present communication, paragraphs of the specification beginning at line 6 on page 43 and at line 15 of page 44 are amended to correct inadvertent errors in the recitation of Figure numbers. In addition, claim 41 is cancelled without prejudice and claims 1, 2, 4, 7, 8, 9, 40, 42 and 46 are amended to define Applicants' invention with greater particularity. The amendments add no new matter, being fully supported by the specification and original claims. Accordingly, claims 1-11, 40, 42, 46 and 47-50 are currently pending and under consideration in this application, with claims 12-39 and 43-45 being withdrawn.

The Drawings

The Office Action indicates that the drawings submitted with the application are objected to for informalities. Applicants submit that formal drawings in which these informalities are addressed will be submitted herein upon allowance of claims.

The Specification

The Office Action indicates that the disclosure is objected to for containing an error in the recitation of "Figure 3" in Example 7, page 44 and appropriate correction is requested. To

PATENT
ATTORNEY DOCKET NO.: UCSD1130-1

Applicants: Fahey, et al.

Application No.: 09/530,370

Filed: July 6, 2000

Page 8

correct the inadvertent error identified by the Examiner, Applicant has amended the Specification to refer to Figure 3 in Example 6, which describes a membrane blot detection of MSH in bacterial colonies and to refer to Figure 4 in Example 7, which describes an ELISA protocol for testing the specificity of an antibody. In view of the amendments to the Specification, Applicants submit the error is corrected and respectfully request reconsideration and withdrawal of the objection to the Specification.

The Rejection under 35 U.S.C. § 112, First Paragraph

Applicant traverses the rejection of claims 1-11, 40-42 and 46-50 under 35 U.S.C. § 112, First Paragraph, as allegedly lacking an enabling disclosure in the Specification. The Examiner alleges in support of the rejection that those of skill in the art could not obtain an antibody that binds to both mycothiol and to precursors of mycothiol without undue experimentation. However, Applicant has amended claims 1, 40 and 46 to require that the invention antibody binds specifically to mycothiol and to thiol-containing mycothiol components. As the Examiner acknowledges (Office Action, page 4), the Examples and Figure 4 in the Specification disclose that an antibody that specifically binds to either mycothiol or to a precursors of mycothiol can be obtained using the procedures described in the Specification. Figure 4 shows that the same antibody that binds to mycothiol binds to the thiol-containing molecule NacCysGlcN, which is a component of mycothiol.

Applicants were the first to actually show that, using the procedures disclosed in the Specification, a purified antibody could be obtained that would bind to either mycothiol or to a

PATENT
ATTORNEY DOCKET NO.: UCSD1130-1

Applicants: Fahey, et al.

Application No.: 09/530,370

Filed: July 6, 2000

Page 9

thiol-containing component of mycothiol, but not to other thiol-containing molecules. In preparation of the invention antibodies, Applicants utilized a protocol designed to ensure that the resultant antibody would bind with specificity to mycothiol and to thiol-containing mycothiol components. As described in Example 2 of the specification, a conjugate of mycothiol and a sulfo-MBS-activated keyhole limpet hemocyanin (KLH) was prepared for use as antigen in immunization of rabbits. MSH-specific antibodies were purified from rabbit sera by affinity chromatography utilizing a structurally dissimilar linker to immobilize MSH to the affinity chromatography column by its sulfhydryl group. Similarly, Example 3 describes preparation of polyclonal antibodies wherein antigen was prepared for immunization with a conjugate of KLH-BS-SM and MSH-specific IgG fractions from rabbit sera were isolated. Specificity for MSH and not for the crosslinker (MBS) or carrier protein (KLH) was detected by a positive reaction to MSH conjugated to ovalbumin via the non-homologous crosslinking agent N-succinimidyl-3-(2-phridyldithio)propionate. As described in Example 7, ELISA for non-specific binding to common biological thiols L-cysteine, glutathione, pantetheine, and coenzyme A produced negative results. However, further ELISAs showed the invention antibody would bind to a thiol-containing component of mycothiol. Moreover, the invention antibody is demonstrated to specifically bind to its antigen in a concentration as low as 10 pmol (See Fig. 4).

Therefore, Applicants respectfully submit that, having once been shown that such antibodies can be obtained using the procedures disclosed in the Examples in the Specification, those of skill in the art would have a reasonable expectation that additional purified antibodies, both monoclonal and polyclonal, could be obtained without undue experimentation that would specifically bind to mycothiol and to thiol-containing components of mycothiol. In particular,

PATENT
ATTORNEY DOCKET NO.: UCSD1130-1

Applicants: Fahey, et al.

Application No.: 09/530,370

Filed: July 6, 2000

Page 10

those of skill in the art would have a reasonable expectation that such an antibody could be obtained against a thiol-containing component of the mycothiol molecule. Thus Applicants respectfully submit that those of skill in the art would not, as the Examiner contends, be required to engage in "undue experimentation" to obtain an antibody that would fall within the scope of the present claims.

Therefore, Applicants respectfully submit that the Specification as supplemented by the skill of the art provides those of skill in the art with sufficient description to provide enablement for the scope of the presently claimed invention. Accordingly, reconsideration and withdrawal of the rejection of claims 1-11, 40-42 and 46-50 under 35 U.S.C. § 112, First Paragraph, are respectfully requested.

PATENT
ATTORNEY DOCKET NO.: UCSD1130-1

Applicants: Fahey, et al.

Application No.: 09/530,370

Filed: July 6, 2000

Page 11

The Rejection under 35 U.S.C. § 102

Applicants respectfully traverse the rejection of claims 1-3, 7 and 8 under 35 U.S.C. 102(b) as allegedly being anticipated by Newton et al. (*Journal of Bacteriology* 178(7):1990-1995, April 1996; hereinafter “Newton”). Applicants submit that the invention method of detecting a member of the taxa actinomycetes, as defined by amended claim 1, distinguishes over the disclosure of Newton by requiring “sequentially incubating a thiol-selective reagent and a purified antibody that specifically binds to mycothiol or a thiol-containing mycothiol component with a sample for a time sufficient for said reagent or said antibody to react with mycothiol or thiol-containing mycothiol component; and detecting reaction of said reagent or said antibody with said mycothiol or said thiol-containing mycothiol component, thereby indicating the presence of a member of the taxa actinomycetes.” Newton fails to provide an enabling disclosure of any type of immunoassay that can detect with specificity mycothiol or the presence of mycothiol in a sample, much less use of an antibody that binds specifically to mycothiol and to thiol-containing components of mycothiol in detection of members of the taxa actinomycetes. Instead, Newton discloses use of the fluorescent reagent monobromobimane (mBBr) for thiol analysis in an HPLC assay of monobromobimane-labeled mycothiol and mentions *in passing* and without any pretense of an enabling description that an antibody-based assay might be developed for developed detection of myothiol in patient samples.

Since Newton fails to provide an enabling disclosure of any immunoassay or use of an antibody in an assay to detect the presence of a member of the taxa actinomycetes, as would be

PATENT
ATTORNEY DOCKET NO.: UCSD1130-1

Applicants: Fahey, et al.

Application No.: 09/530,370

Filed: July 6, 2000

Page 12

required to establish anticipation of present claims 1-3, 7 and 8 under 35 U.S.C. § 102(b),

Applicants submit that anticipation is not established by the reference. Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

The Rejection under 35 U.S.C. § 103(a)

Applicant respectfully traverses the rejection of claims 40 and 42 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Newton. Applicants disagree with the Examiner's assertion that "it would have been obvious at the time the invention was made ... to package the reagents utilized in the methods of Newton et al. into a convenient kit form" (Office Action, page 6). Claim 41 has been canceled by the present communication, thus rendering the rejection moot as to claim 41. Applicants respectfully submit that the invention kits useful for detecting the presence of mycothiol or thiol-containing mycothiol component in a sample, as defined by amended claim 40, distinguish over Newton by requiring the kit to comprise "carrier means being compartmentalized to receive in close confinement therein one or more containers comprising a container containing an antibody specific for mycothiol or a thiol-containing mycothiol component to detect mycothiol or the thiol-containing mycothiol component in the sample."

By contrast, Newton is absolutely silent regarding an antibody that binds specifically to mycothiol or a thiol-containing mycothiol component. Instead, Newton discloses the chemical reagent monobromobimane, which is used in an HPLC assay by chemical reaction with the thiol present in the molecule and Newton provides no disclosure of a procedure for obtaining an

PATENT
ATTORNEY DOCKET NO.: UCSD1130-1

Applicants: Fahey, et al.

Application No.: 09/530,370

Filed: July 6, 2000

Page 13

antibody that binds specifically to mycothiol or a thiol-containing mycothiol component (i.e., that does not bind to other thiol-containing molecules in the sample).

Moreover, Applicants submit that there is no suggestion in Newton how to modify the disclosed method of HPLC detection of a chemically modified mycothiol to provide an immunoassay employing an antibody specific for mycothiol or a thiol-containing mycothiol component. That is, there is no suggestion in Newton how to substitute an anti-mycothiol antibody for the chemical reagent monobromobimane for use in an immunoassay to detect the presence of mycothiol or thiol-containing mycothiol components because anti-mycothiol antibodies were unknown in the art at the filing date of the present application.

Moreover, mycothiol is a small molecule that is not immunogenic. Preparation of antibodies to mycothiol had never been described in the art and those of skill in the art would not have had a reasonable assurance that an antibody could be obtained that would be specific for both mycothiol and for thiol-containing mycothiol components, such as thiol-containing biological precursors of mycothiol. The discussion above regarding the elaborate procedure Applicants employed to obtain the invention antibodies applies equally here. Applicants submit that such a procedure would not have been obvious to those of skill in the art at the filing of the application.

Furthermore, Applicants disclose that the invention anti-mycothiol antibody, when used in an ELISA format assay, provides a result that is surprisingly more accurate than was disclosed in the reference. For example, Applicants teach that the invention antibody, when used in an ELISA format to detect the presence of mycothiol in a sample, has a useful range of about 0.1 - 1

PATENT
ATTORNEY DOCKET NO.: UCSD1130-1

Applicants: Fahey, et al.

Application No.: 09/530,370

Filed: July 6, 2000

Page 14

pmol MSH, and a detection limit of at least 0.3 fmol MSH.” This result allows for an immunoassay that is “approximately 4 orders of magnitude more sensitive” than the HPLC assay disclosed in the reference (Specification, page 52, final line to page 53, line 4). Therefore, Applicants respectfully submit that there is no suggestion in Newton’s disclosure how to prepare the invention kit, as defined by amended claim 40, that would be useful for an immunoassay specifically to detect mycothiol or presence thereof in a sample.

Accordingly, Applicants respectfully submit that the invention kits, as defined by present claims 40-42, are not rendered unpatentable under 35 U.S.C. 103(a) by Newton and reconsideration and withdrawal of the rejection are respectfully requested.

Claim Objection

The Office Action indicates that claim 2 is objected to as containing an informality, namely the misspelling of “actinomycetes.” By the present communication, claim 2 has been amended to correct the inadvertent error in the spelling of “actinomycetes” and reconsideration and withdrawal of the rejection are respectfully requested.

In view of the above amendments and remarks, Applicants submit that all rejections and objections have been overcome. Reconsideration and favorable action on all claims are respectfully requested. If the Examiner would like to discuss any of the issues raised in the

PATENT
ATTORNEY DOCKET NO.: UCSD1130-1

Applicants: Fahey, et al.
Application No.: 09/530,370
Filed: July 6, 2000
Page 15

Office Action, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

Date: August 19, 2002

June M. Learn

June M. Learn
Registration No.: 31,238
Telephone: (858) 677-1416
Facsimile: (858) 677-1465

USPTO CUSTOMER NUMBER 28213
GRAY CARY WARE & FREIDENRICH LLP
4365 Executive Drive, Suite 1100
San Diego, California 92121-2133

Enclosure: Exhibit A

PATENT
ATTORNEY DOCKET NO.: UCSD1130-1

Applicants: Fahey, et al.

Application No.: 09/530,370

Filed: July 6, 2000

Exhibit A: Page 1

EXHIBIT A

Version with Markings to Show Changes Made

Please amend the paragraph beginning at line 6 on page 43 to read as follows:

A membrane-based assay based on the immunoassay architecture described in Example 4 was developed for detecting MSH production by bacterial colonies grown on solid media. The following protocol is typical for a single 100 mm Petri dish. All incubations were done at room temperature on an orbital platform shaker set to 90 rpm unless otherwise noted. Different bacteria, including two strains of *Mycobacterium smegmatis* (mc²-6 and mc²-155) and several non-MSH-producing species (*Escherichia coli* HB101, *Enterococcus faecalis* ATCC 29212, and *Streptococcus mutans* ATCC 33402) were grown as separate streaks on a single agar dish. A supported nitrocellulose membrane circle ([*"NitroPure"*] **NITROPURE®**, 0.45 µm porosity, 81 mm diameter) was marked with a pencil for orientation on the plate, and pre-soaked in TBS. Excess TBS was drained from the membrane, a freshly-made solution of Pierce [Imject] **IMJECT®** maleimide-activated BSA (265 µg in 13.3 ml TBS, to give 5 µg/cm² loading) added, and the membrane incubated for 30 min. Excess liquid was drained from the membrane, which was then laid onto the surface of the bacterial plate with care to avoid bubbles or smearing of the bacterial colonies. The membrane was lifted carefully and laid bacteria-side up for 1 h in a clean glass Petri dish containing a solution of *N*-acetylglucosaminidase (3.1 units in 10 ml TBS adjusted to pH 4.2 with acetic acid). The membrane was next washed briefly with TBS to remove adhering cells and washed with 10 ml TBST. Excess liquid was drained and the membrane incubated in 10 ml 2% fish skin gelatin in TBS for 2 h. The membrane was drained,

PATENT
ATTORNEY DOCKET NO.: UCSD1130-1

Applicants: Fahey, et al.

Application No.: 09/530,370

Filed: July 6, 2000

Exhibit A: Page 2

10 ml affinity-purified anti-MSH IgG solution (containing ~18 µg total protein) added and incubated overnight at 4 °C on an orbital platform shaker set to 60 rpm. The antibody solution was aspirated and the membrane washed 3 times in TBST (10 ml and 10 min for each wash). Excess liquid was drained, 10 ml of secondary antibody (goat anti-rabbit [whole IgG] F(ab')₂ fragments conjugated to bovine intestinal alkaline phosphatase, diluted 1:15000 in TBS) added, and the membrane incubated for 1 h. The membrane was drained, washed twice in TBST and thrice in TBS (10 ml and 5 min each wash). Development was with BCIP-NBT (SigmaFAST®). After thoroughly washing in distilled water, the blot was air-dried. MSH-containing bacteria are revealed as dark purple stains; only the two strains of *M. smegmatis* produced positive signals (Figure 3).

Please amend the two paragraphs that begin at line 15 of page 44 to read as follows:

Since reaction of typical cellular constituents with maleimide-BSA is largely limited to thiols, it was considered important to ascertain whether any typical biological thiols would produce false-positive assay results. The following thiols were tested in the ELISA protocol described above in Example 4: L-cysteine, glutathione, pantetheine, and coenzyme A. At the 10 pmol level all of these gave negative results indicating that these are not recognized by the anti-mycothiol antibody (Figure [3] 4).

In order to test whether the anti-mycothiol antibody recognizes component parts of the mycothiol molecule we also tested the ELISA on *N*-acetyl-L-cysteine, L-cysteinyl-glucosamine, (([2-L-cysteinyl][2]-amido-2-deoxy- α -D-glucopyranose), and *N*-acetyl-L-cysteinyl-glucosamine

PATENT
ATTORNEY DOCKET NO.: UCSD1130-1

Applicants: Fahey, et al.

Application No.: 09/530,370

Filed: July 6, 2000

Exhibit A: Page 3

((2-N-acetyl-L-cysteinyl)[-2-]amido-2-deoxy- α -D-glucopyranose). The first two gave negative results at the 10 pmol level but the latter compound, which has the structure of MSH with the inositol removed, gave a positive assay with a sensitivity of about 4% of that for MSH (Figure [3] 4).

In the Claims

Please cancel claim 41 without prejudice.

Please amend claims 1, 2, 4, 7, 8, 9, 40, 42 and 46 as follows:

1. (Amended) A method of detecting a member of the taxa actinomycetes, comprising
 - (a) sequentially incubating a thiol-selective reagent and a purified antibody that [detects] specifically binds to mycothiol or a [precursor thereof] thiol-containing mycothiol component with a sample for a time sufficient for said reagent or said antibody to react with mycothiol or [precursor thereof] a thiol-containing mycothiol component; and
 - (b) detecting [said] reaction of said reagent or said antibody with said mycothiol or [a precursor thereof] said thiol-containing mycothiol component,
[wherein detection of a reaction is indicative of] thereby indicating the presence of a member of the taxa actinomycetes.

PATENT
ATTORNEY DOCKET NO.: UCSD1130-1

Applicants: Fahey, et al.

Application No.: 09/530,370

Filed: July 6, 2000

Exhibit A: Page 4

2. (Amended) The method of claim 1, wherein said member of the taxa [actinomyces] actinomycetes is mycobacteria.

4. (Amended) The method of claim 1, wherein [said reagent is] said antibody is incubated with the sample before said thiol-selective reagent.

7. (Amended) The method of claim 1, further comprising

(c) quantitating said mycothiol or [precursor thereof] said thiol-containing mycothiol component.

8. (Amended) The method of claim 1, wherein said [precursor] thiol-containing mycothiol component is selected from [the group consisting of 1-D-*myo*-inosityl-2-amino] 2-(N-acetylcysteinyl)amido-2-deoxy- α -D-glucopyranose and 1D-*myo*-inosityl-2-(L-cystinyl)cysteinyl)amido-2-deoxy- α -D-glucopyranoside.

9. (Amended) An antibody which binds specifically to mycothiol or a thiol-containing mycothiol [precursor] component.

40. (Amended) A kit useful for detecting the presence of mycothiol or [precursor thereof] thiol-containing mycothiol component in a sample, the kit comprising: carrier means being compartmentalized to receive in close confinement therein one or more containers comprising a container containing [a reagent] an antibody specific for mycothiol or a thiol-containing mycothiol component to detect mycothiol or [presence thereof] thiol-containing mycothiol component in the sample.

PATENT
ATTORNEY DOCKET NO.: UCSD1130-1

Applicants: Fahey, et al.

Application No.: 09/530,370

Filed: July 6, 2000

Exhibit A: Page 5

42. (Amended) The kit of claim 40, further comprising a container containing a detection reagent to detect the reaction of the mycothiol or [precursor thereof] thiol-containing mycothiol component with said [reagent] antibody to detect mycothiol or [precursor thereof] thiol-containing mycothiol component.

46. (Amended) The method of claim 1, wherein the thiol-selective reagent [is] comprises biotin and biotinylated mycothiol or biotinylated thiol-containing mycothiol [precursor] component is formed in (a); and wherein said detecting in (b) comprises contacting the biotinylated mycothiol or [biothinylation] biotinylated thiol-containing mycothiol [precursor] component with a primary antibody that binds [thereto] to mycothiol or the thiol-containing mycothiol component to form a complex and detecting the presence of said complex with a detection reagent that binds to biotin.